भारतीय मानक
Indian Standard

IS 10633 : 2017
(Reaffirmed 2022)

वनस्पति — विशिष्टि

(तीसरा पुनरीक्षण)

Vanaspati — Specification

(Third Revision)

ICS 67.200

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September 2017 **Price Group 8**

Oils and Oilseeds Sectional Committee, FAD 13

FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Vanaspati is one of the items of the mass consumption in the country, therefore, it is desirable that proper quality control is exercised in the production of the product in order to provide wholesome and safe product. Keeping this in view this Indian Standard was first published in 1983 and was subsequently revised in 1986. In the second revision published in 1999, the packing and other clauses were modified to align with the provision of *Prevention of Food Adulteration Act*, 1954 and Rules framed therein and the scheme for labelling environment friendly products to be known as ECO-Mark was introduced at the instance of the Ministry of Environment and Forests (MEF).

This revision has been carried out to harmonize the standard with *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Vegetable Oils Grading and Marking Rules*, 1955.

In this revision the following major changes have been made:

a) Limit of aflatoxin has been prescribed;
b) Aflatoxin is determined using High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) instead of Thin Layer Chromatography (TLC) prescribed earlier; and
c) Limits of trans fatty acids has been incorporated and the requirement of melting point has been removed to align with *Food Safety and Standards Act*, 2006 and Regulations framed thereunder.

In the formulation of this standard, due consideration has been given to *Food Safety and Standards Act*, 2006 and Regulations framed thereunder; *Legal Metrology Act*, 2009 and Rules framed thereunder and the *Essential Commodities Act*, 1955. However, this standard is subject to restrictions imposed under these, wherever applicable.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

VANASPATI — SPECIFICATION

(Third Revision)

1 SCOPE

This standard prescribes requirements and methods of sampling and test for *Vanaspati*.

2 REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
75 : 2014	Linseed oil — Specification (*third revision*)
170 : 2004	Acetone — Specification (*fourth revision*)
323 : 2009	Rectified spirit for industrial use — Specification (*second revision*)
542 : 2014	Coconut oil — Specification (*third revision*)
543 : 2014	Cottonseed oil — Specification (*third revision*)
544 : 2014	Groundnut oil — Specification (*third revision*)
545 : 2014	*Mahua* oil — Specification (*fourth revision*)
546 : 2014	Mustard oil — Specification (*third revision*)
547 : 2014	Sesame oil — Specification (*third revision*)
548	Methods of sampling and test for oils and fats:
(Part 1) : 1964	Methods of sampling, physical and chemical tests (*revised*)
(Part 2) : 1976	Purity tests (*third revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
1699 : 1995	Methods of sampling and test for food colours (*second revision*)
3448 : 2014	Rice bran oil — Specification (*third revision*)
3490 : 2014	Nigerseed oil — Specification (*first revision*)
3491 : 2014	Safflower seed oil — Specification (*first revision*)
4055 : 2014	Maize (Corn) oil — Specification (*first revision*)
4276 : 2014	Soybean oil — Specification (*second revision*)
4277 : 2014	Sunflower oil — Specification (*second revision*)
5637 : 1970	Specification for watermelon seed oil
7375 : 1979	Specification for salseed fat (*first revision*)
8323 : 2014	Palm oil — Specification (*first revision*)
8361 : 2014	Palmolein — Specification (*first revision*)
8879 : 1980	Specification for *Dhupa* fat (*first revision*)
8591 : 1980	Specification for *Kokum* fat (*first revision*)
9231 : 1979	Specification for mango kernel fat
10325 : 2000	Square tins — 15 kg/litre for ghee, *VANASPATI*, edible oils and bakery shortenings — Specification (*second revision*)
10840 : 1994	Blow moulded HDPE containers for packing of *VANASPATI* — Specification (*second revision*)
11352 : 1998	Flexible packs for the packing of *VANASPATI* upto 5 kg or 5 litres — Specification (*second revision*)
11476 : 1985	Glossary of terms relating to oils and fats
14349 : 1996	Code for hygienic conditions for edible oil and *VANASPATI* manufacturing units
IS/ISO 14718 : 1998	Animal feedings stuffs — Determination of aflatoxin B1 content of mixed feeding stuffs — Method using high performance liquid chromatography

3 DEFINITION

For the purpose of this standard, the definitions given in IS 11476 and also the following shall apply.

3.1 ***Vanaspati*** — *Vanaspati* means any refined edible vegetable oil or oils, subjected to a process of hydrogenation in any form or chemical or enzymatic inter-esterification. It shall be prepared by

hydrogenation from groundnut oil, cottonseed oil and sesame oil or mixtures thereof or any other harmless vegetable oils allowed by the government for the purpose. Refined sal seed fat, if used, shall not be more than 10 percent of the total oil mix.

4 PROCESS

The material shall be prepared by process of hydrogenation in any form.

5 RAW MATERIAL

5.1 Only vegetable oil, and in such proportions as specified from time to time by the *Food Safety and Standards Act*, 2006 and Regulations framed thereunder shall be used. As on date, preparation of *Vanaspati* from the following vegetable oils is permitted:

a) Coconut oil (*see* IS 542),
b) Cottonseed oil (*see* IS 543),
c) *Dhupa* fat (*see* IS 8879),
d) Groundnut oil (*see* IS 544),
e) *Kokum* fat (*see* IS 8591),
f) Linseed oil (*see* IS 75),
g) *Mahua* oil (*see* IS 545),
h) Maize (Corn) oil (*see* IS 4055),
j) Mango kernel fat (*see* IS 9231),
k) Mustard/Rape-seed oil (*see* IS 546),
m) Nigerseed oil (*see* IS 3490),
n) Palm oil (*see* IS 8323),
p) Phulwara fat,
q) Rice bran oil (*see* IS 3448),
r) Safflower (Kariseed) oil (*see* IS 3491),
s) Salseed oil (up to 10 percent) (*see* IS 7375),
t) Sesame oil (*see* IS 547),
u) Soyabean oil (*see* IS 4276),
v) Sunflower oil (*see* IS 4277),
w) Watermelon seed oil (*see* IS 5637),
x) Vegetable oils imported for edible purposes, and
y) Palm stearin.

5.2 The material shall contain such quantity of refined sesame oil [*see* IS 547] which is sufficient to ensure that when the material is mixed with refined groundnut oil [*see* IS 544] in the proportion of 20 : 80, the red colour produced by the Baudouin test shall not be lighter than 2 red units in 1 cm cell on Lovibond scale when tested in accordance with the method given in Annex A.

5.3 The material shall be free from non-edible oils, mineral oil, castor oil and animal fats when tested by the methods given in **9**, **10**, **12**, **14**, **15** and **18** of IS 548 (Part 2).

5.4 Only those chemicals which can be totally removed and shall have no adverse effect on quality of the product shall be used in the processing.

6 REQUIREMENTS

6.1 Colour

The *Vanaspati* shall be of white to pale yellow in colour.

6.2 Appearance

The material shall be clear in appearance on melting. The clarity of the material shall be judged by the absence of turbidity after heating the sample to 70 ± 0.5°C and keeping for 1 h at this temperature.

6.3 The material shall be free from sediments, suspended and other foreign matter separated water, added colouring or flavouring substances or any other substances deleterious to health.

6.4 Odour and Taste

The material shall have a characteristic odour and taste shall be free from rancidity or staleness, foreign odour and taste.

6.5 Anti-oxidant

No anti-oxidant, synergist, emulsifier or any other substance shall be added to it except with the prior sanction of the Food Safety and Standards Authority.

Provided that imported crude palm oil and fractions thereof shall not be used by the producers other than those who are engaged in manufacture of *Vanaspati* / any other hydrogenated oil produce and are equipped in the same location with the facilities for generation of hydrogen gas and hydrogenation of the said imported crude palm oil and fractions thereof with the gas so generated in the manufacture of *Vanaspati*/any other hydrogenated vegetable oil product for edible consumption.

Diacetyl to the extent of 4 mg/kg may be added to *Vanaspati* exclusively meant for consumption by the Armed Forces.

6.6 Oils shall not contain aflatoxin, more than 30 µg/kg, when tested by the method prescribed in IS/ISO 14718 or as prescribed in Annex B.

6.7 Metal contaminants and pesticide residues shall not exceed the tolerance limits as prescribed in the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011.

6.8 The material shall be manufactured in the premises maintained under hygienic conditions (*see* IS 14349).

6.9 The material shall also conform to the requirements given in Table 1.

Table 1 Requirements for *Vanaspati*

(*Clause* 6.9)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Moisture, percent by mass, *Max*	0.25	**5** of IS 548 (Part 1)
ii)	Acid value, *Max*	0.5[1)]	**7** of IS 548 (Part 1)
iii)	Unsaponifiable matter[2)], percent by mass, *Max*	2.0	**8** of IS 548 (Part 1)
iv)	Refractive index at 60°C, *Min*	1.450 5	**10** of IS 548 (Part 1)
v)	Synthetic vitamin A, expressed in international units (I. U.) per gram:		
	a) When packed, *Min*	25	Annex C
	b) At retail outlet	Shall pass the test	Annex D
vi)	Nickel, mg/kg, *Max*	1.5	Annex E
vii)	Trans fatty acids, percent by weight, *Max*	5.0	Annex F

[1)] This corresponds to free fatty acids (FFA) when expressed as oleic acid percent by mass, *Max* of 0.25.

[2)] In case of *vanaspati* where proportion of rice bran oil is more than 30 percent by mass, the unsaponifiable matter, percent by mass, *Max* shall be 2.5 percent provided the quantity of rice bran oil of such *vanaspati* is declared on the label.

7 OPTIONAL REQUIREMENTS FOR ECO-MARK

7.1 General Requirements

7.1.1 The product shall conform to the requirements of quality as given in **5** and **6**.

7.1.2 The manufacturers shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the norms laid down under the *Water* (*Prevention and Control of Pollution*) *Act*, 1974; *Air* (*Prevention and Control of Pollution*) *Act*, 1981; *Water* (*Prevention and Control of Pollution*) *Cess Act*, 1977 respectively, alongwith the authorization, if required, under the *Environment* (*Protection*) *Act*, 1986, while applying for ECO BIS Mark.

7.2 Specific Requirements

7.2.1 The nickel content of the product shall not exceed 0.5 mg/kg when tested by the atomic absorption spectrometric method prescribed in Annex E.

7.2.2 The product shall be free from obnoxious chemicals.

7.2.3 The product shall not contain any of the toxic metals in excess of the quantities prescribed in Table 2.

Table 2 Limits for Toxic Metals

(*Clause* 7.2.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Lead, mg/kg, *Max*	0.5	**15** of IS 1699
ii)	Arsenic, mg/kg, *Max*	0.5	do
iii)	Cadmium, mg/kg, *Max*	1.0	do
iv)	Mercury (total) mg/kg, *Max*	0.25	do

8 PACKING

8.1 The material shall be packed in suitable sealed cartons or flexible packs (*see* IS 11352) or plastic containers (*see* IS 10840) or tin containers (*see* IS 10325).

8.2 For ECO-Mark, the product shall be packed in such packages which are made from recyclable (that is which can be re-processed to manufacture any useful product) or biodegradable materials.

9 MARKING

9.1 The containers shall be marked in English or Hindi in *Devnagri* script with the following information:

a) Name/description of the contents;
b) Net quantity;
c) Words ‘contains 25 I.U. of vitamin A per gram, when packed’;
d) Name and percentage of antioxidants added, if any;
e) Manufacturers name, his recognized trademark, if any and address;
f) Batch number or lot number in code or otherwise;
g) Month and year of manufacture;
h) Shelf life, best before (month and year);
j) MADE FROM VEGETABLE OILS ONLY provided on the label affixed to the container or lithographed or stenciled thereon with indelible ink of type of size of not less than 12.5 mm in case of 15 kg/l5 litres or above tin containers;
k) Contains trans fatty acids, ____ percentage by weight;
m) Free from Argemone Oil;

n) *Nutritional information* — Nutritional information or nutritional facts per 100 g or 100 ml or per serving of the product shall be given on the label containing the following:

1) energy value, in kcal;
2) the amounts of protein, carbohydrate (specify quantity of sugar) and fat, in gram (g) or ml;
3) the amount of any other nutrient for which a nutrition or health claim is made:

Provided that where a claim is made regarding the amount or type of fatty acids or the amount of cholesterol, the amount of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, in gram (g), and cholesterol, in milligram (mg), shall be declared, and the amount of trans fatty acid, in gram (g), shall be declared in addition to the other requirement stipulated above.

p) Every package of *vanaspati* made from more than 30 percent of Rice bran oil shall bear the following label:

This package of *vanaspati* is made from more than 30 per cent Rice bran oil by weight

q) Any other requirement as stipulated under *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Legal Metrology Act*, 2009 and rules framed thereunder.

9.2 For ECO-Mark, the container shall be marked with the following in addition to those given under **9.1**:

a) List of identified critical ingredients in descending order of quantity, percent by mass; and
b) The brief criteria for which the product has been labelled for ECO-Mark.

9.3 BIS Certification Marking

The product may also be marked with the Standard Mark.

9.3.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

9.4 ECO-Mark

The product may also be marked with the ECO-Mark, the details of which may be obtained from Bureau of Indian Standards.

10 SAMPLING

Representative samples of the material shall be drawn as given in **3** of IS 548 (Part 1).

ANNEX A

(*Clause* 5.2)

TEST FOR THE PRESENCE OF SESAME OIL (BAUDOUIN TEST)

A-0 GENERAL

A-0.1 Outline of the Method

The development of a permanent pink colour with furfural solution in presence of hydrochloric acid indicates the presence of sesame oil.

A-1 REAGENTS

A-1.1 Refined Groundnut Oil showing negative Baudouin test [*see* IS 544].

A-1.2 Dilute Hydrochloric Acid, relative density 1.125.

A-1.3 Concentrated Hydrochloric Acid, fuming, relative density 1.19.

A-1.4 Furfural Solution, 2 percent solution of furfural, distilled not earlier than 24 h prior to the test, in rectified spirit (*as per* IS 323). The reagent is stable up to 3 months if kept in a refrigerator.

A-2 APPARATUS

A-2.1 Lovibond Tintometer

A-2.2 Measuring Cylinder, 25 ml capacity.

A-3 PROCEDURE

A-3.1 Melt the sample of the vegetable oil product, that is, *Vanaspati* completely and mix it well at a temperature of about 50°C.

A-3.2 Check for the presence of colouring matter which are chromogenic in the presence of hydrochloric acid, by the method given in **A-3.2.1** and **A-3.2.2**.

A-3.2.1 Shake 10 ml of the melted *Vanaspati* with 10 ml concentrated hydrochloric acid. Note if any red colour develops in the aqueous layer.

A-3.2.2 If a red colour develops in the aqueous layer, shake 20 ml of the melted vegetable oil product in a separating funnel for 30 s with 15 ml of dilute hydrochloric acid. During treatment, do not permit the temperature of the contents of the separating funnel to exceed that temperature necessary to keep the sample in liquid condition. Draw off the red acid layer which collects at the bottom of the funnel and repeat the process until no further colouration takes place.

A-3.3 Dilute 20 ml of the melted vegetable oil product (that is, take the original sample, if no red colour develops on checking as in **A-3.2.1**, otherwise take the product after complete removal of the hydrochloric acid layer as in **A-3.2.2**) with 80 ml of refined groundnut oil. Take 5 ml of this mixture in a 25 ml measuring cylinder with glass stopper and add 5 ml of concentrated hydrochloric acid. Add 0.4 ml of the Furfural solution, shake vigorously for two minutes, and allow to stand for 5 minutes. Transfer the contents of the measuring cylinder to a separating funnel and decant the acid layer through a wet filter paper into a cleaned (with carbon tetrachloride) and dried 1 cm Lovibond cell. Place the cell in position in a Lovibond Tintometer and viewing through the eyepiece of the instrument, match the colour shade of the filtrate with the appropriate combination of red and yellow slides. The colour, which is recorded in terms of red units only (correct to one place of decimal) shall be read within 12 min of the addition of furfural solution.

A-3.4 Perform a blank experiment, using 5 ml of refined groundnut oil in place of the mixture and determine the colour. The Baudouin test reading of the sample given shall be the colour reading given by the sample as in A-3.3 subtracted by the colour reading given by the blank experiment.

ANNEX B

(*Clause* 6.6)

DETERMINATION OF TOTAL AFLATOXIN BY ELISA

B-1 PRINCIPLE

Antibodies specific to aflatoxins B1, B2 and G1 are immobilized on the filter, and toxin (aflatoxin B1) is labelled with an enzyme (horseradish peroxidase). Binding of toxin-enzyme conjugate by immobilized antibodies is inhibited by addition of free toxin present in the test sample. Bound enzyme catalyses oxidation of substrate to form a blue complex. Development of colour indicates that the test sample contains aflatoxin.

B-2 APPARATUS

B-2.1 Antibody Coated Solid Support

B-2.2 Aflatoxin Enzyme Conjugate

B-2.3 High Speed Blender

B-2.4 Variable 100-1 000 µl Micropipettes

B-2.5 Glass Culture Tubes

B-2.6 Filters

B-2.7 Timer

B-2.8 Silicon Carbide Boiling Chips

B-3 REAGENTS

B-3.1 Wash Solution-Phosphate Buffered Saline Solution — Dissolve 0.23 g $NaH_2PO_4.H_2O$, 1.95 g $K_2HPO_4.3H_2O$, 8.70 g NaCl, 0.125 ml Tween 20 and 10 mg thimerosal in 900 ml H_2O, adjust *p*H to 7.2 and dilute to 1 litre.

B-3.2 Buffer — 0.1 percent Bovine serum albumin in phosphate buffer saline solution containing 0.05 percent thimerosal.

B-3.3 Substrate Solution A, tetramethylbenzidine (TMB), (0.4 g/l H_2O), *p*H 8.3.

B-3.4 Substrate Solution B, hydrogen peroxide (0.02 percent H_2O_2 in 0.13 percent aq. Citric acid solution, *p*H 3.0).

B-3.5 Methanol

B-3.6 Hexane

B-3.7 Chloroform

B-3.8 NaH_2PO_4

B-3.9 K_2HPO_4

B-3.10 NaCl

B-3.11 Tween 20

B-3.12 Bovine Serum Albumin

B-4 PROCEDURE

B-4.1 Preparation of Sample

B-4.1.1 Weigh 50 g of sample into blender jar.

B-4.1.2 Mix with 250 ml of 55 percent methanol and 45 percent water (*see* IS 1070).

B-4.1.3 Mix 100 ml hexane and blend for 1 min at high speed.

B-4.1.4 Filter mixture and recover filtrate.

B-4.1.5 Leave for 5 min and remove the lower phase containing methanol water (*see* **B-4.1.2**).

B-4.2 Testing

B-4.2.1 Bring all reagents at room temperature (20-23°C).

B-4.2.2 Prepare fresh substrate in small culture tubes by mixing 500 µl substrate solution A with 500 µl substrate solution B for each reaction sites used.

B-4.2.3 Add 100 µl test extract to 200 µl buffer (*see* **B-3.2**).

B-4.2.4 Thoroughly mix the diluted test extract and apply 100 µl diluted test extract to the centre of membrane. Using timer, wait for 1 min.

B-4.2.5 Apply 100 µl (2 drops) enzyme solution to the centre of membrane. Using timer, wait for 1 min.

B-4.2.6 Wash with 1.5 ml (30 drops) wash solution added drop-wise.

B-4.2.7 Add the entire content of the substrate solution 1.0 ml from each test tube to each reaction site. Wait 1 min and immediately observe site (centre of cup) for blue colour development (negative) or no colour development (positive).

B-4.3 Interpretation of Results

A-4.3.1 Observe the reaction site (centre of the cup) for a blue colour or no colour development at exactly 1 min after adding the substrate A and B mixture (*see* **B-3.3** and **B-3.4**).

Negative — If the reaction site (centre of the cup) turns light blue or darker, test sample contains total aflatoxin B1, B2 and G1.

Positive — If no blue colour is observed in the reaction site (centre of cup) and reaction site remains completely white (no colour change) for at least 1 min, the test sample contains aflatoxin B1, B2 and G1.

ANNEX C

[*Table* 1, *Sl No.* (v) (a)]

DETERMINATION OF VITAMIN A

C-1 GENERAL

C-1.1 Principle

The content of vitamin A is determined by measurement of the ultra-violet absorption spectrum of a fraction, in which the vitamin A alcohol is collected after its isolation by chromatography. It is expressed in international units (I.U.) per gram.

C-1.1.1 One international unit (I.U.) of vitamin A is equivalent to 0.3 mg vitamin A alcohol or 0.344 mg vitamin A acetate.

NOTE — The official method in use in the United Kingdom (UK) involves a somewhat different procedure to that given in this method, but both techniques give the same result for vitamin A.

C-2 REAGENTS

C-2.1 Potassium Hydroxide, 50 percent aqueous solution.

C-2.2 Ethanol, 96 percent (*v/v*).

C-2.3 Diethyl Ether, peroxide-free.

NOTE — Make the diethyl ether peroxide-free by distillation over potassium hydroxide. Store the peroxide-free ether over coarse granular carbon.

C-2.4 Petroleum Ether, distilled over potassium hydroxide, boiling range 40 to 60°C.

C-2.5 Alumina (Activated) — Heat the alumina at 600°C for 6h, cool, sieve through a 180 mesh sieve, and. dd about 3 percent water. Mix thoroughly and allow the product to stand for at least 12 h, before use. Store in an air tight bottle.

C-2.6 Alumina (Alkaline) — The same alumina as given in **C-2.5** is treated with sodium hydroxide as follows:

Stir 10 g alumina with a solution of a solution of 1 g sodium hydroxide in 10 ml water. Allow to stand at room temperature for 1 h in a closed bottle, and shake occasionally. Then heat in a dish in a vacuum drying oven at 100°C and 20 mm Hg for 2 h 30 min. Pour the dried product into a bottle without removing any powder clinging to the wall of the dish, and stopper securely. To the cooled powder, add 2 ml water and mix thoroughly. Allow to stand for 18 h and determine the moisture content after drying for 1 h in a drying oven at 105°C; if lower than 2 percent, again add water, mix and repeat the process.

C-2.7 Antimony Trichloride Solution — With a porcelain spoon, introduce 65 g of antimony trichloride into a 500 ml conical flask and wash several times with 15 ml chloroform until the chloroform remains clear. Then dissolve the antimony trichloride in 200 ml of chloroform by refluxing. Transfer the warm solution to a bottle containing anhydrous sodium sulphate.

After some days, antimony trichloride crystals are formed at the bottom and the wall; the solution is then quite clear and ready for use.

NOTE — The chloroform to be used should be washed with water and dried before use, to remove any alcohol which may have been added to improve keep ability.

C-2.8 Acetone, as per IS 170.

C-3 APPARATUS

C-3.1 Conical Flask, 50 ml.

C-3.2 Separating Funnels, 500 ml.

C-3.3 Round Bottom Flask, 200 ml with ground glass stopper, and two condensers.

C-3.4 Chromatographic Apparatus — The apparatus consists of two parts, each containing a chromatographic column, which may readily be connected in series by means of a rubber stopper connection (*see* Fig. 1). The upper tube contains alumina (*see* Fig. 2), while the lower tube contains alumina (alkaline) (*see* Fig. 3). Close both the tubes at the bottom with a plug of cotton wool. Fill the longer tubes with petroleum ether to a level which reaches into the widened section, and then very regularly and gently pour out the alumina to a column height of 15 cm. Fill the shorter, column, shortly before use, in the same way to a height of 2 cm with alkaline alumina.

C-3.5 Tubes, calibrated at 1 ml (*see* Fig. 4).

C-3.6 Pipette, 1 ml with a fine tip.

C-3.7 Graduated Flask, 10 ml.

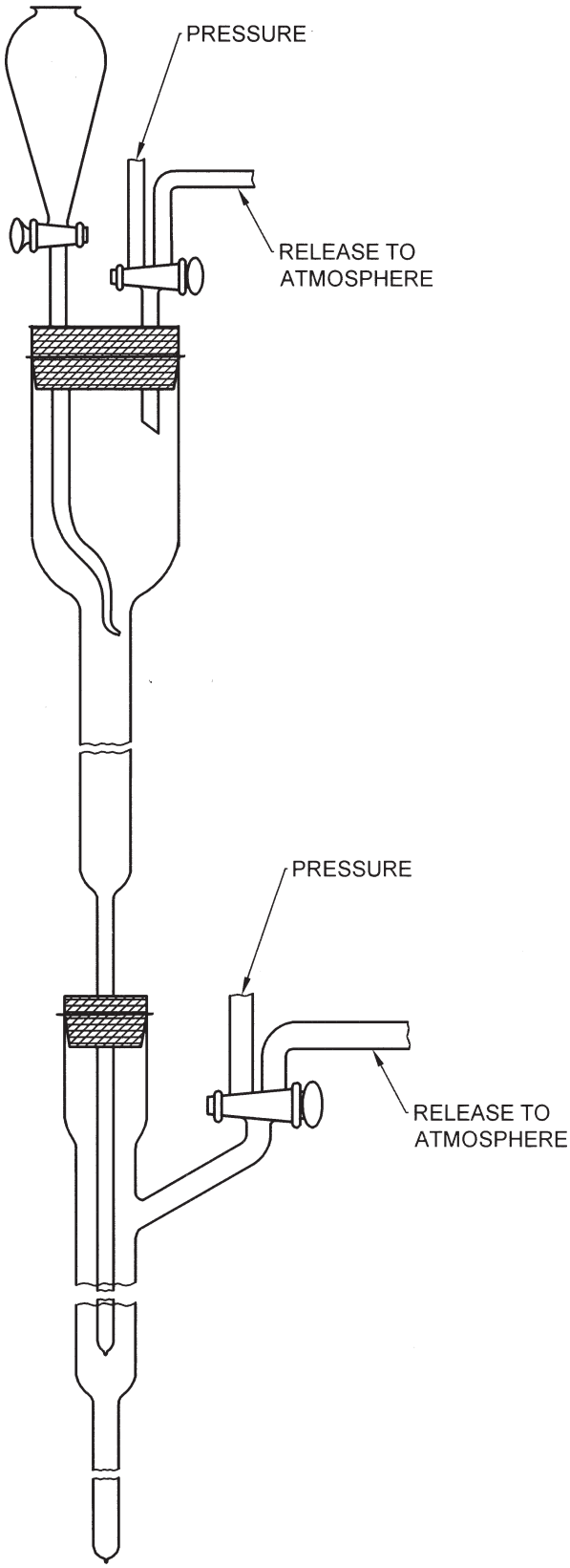
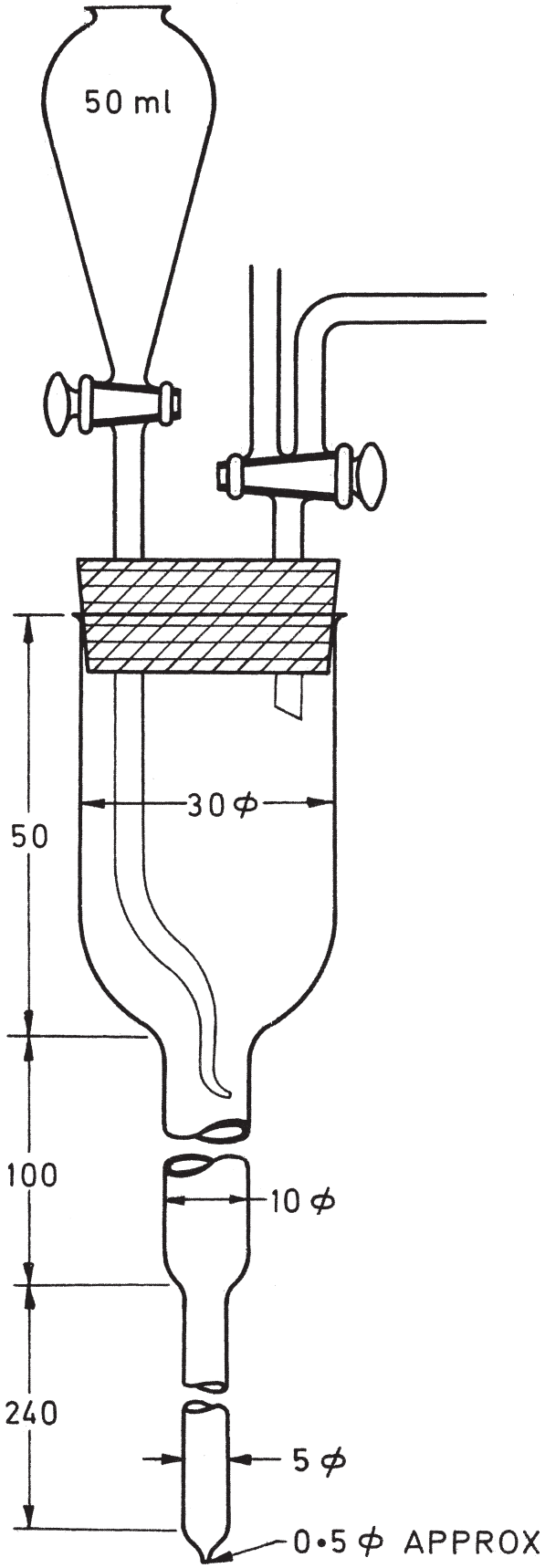
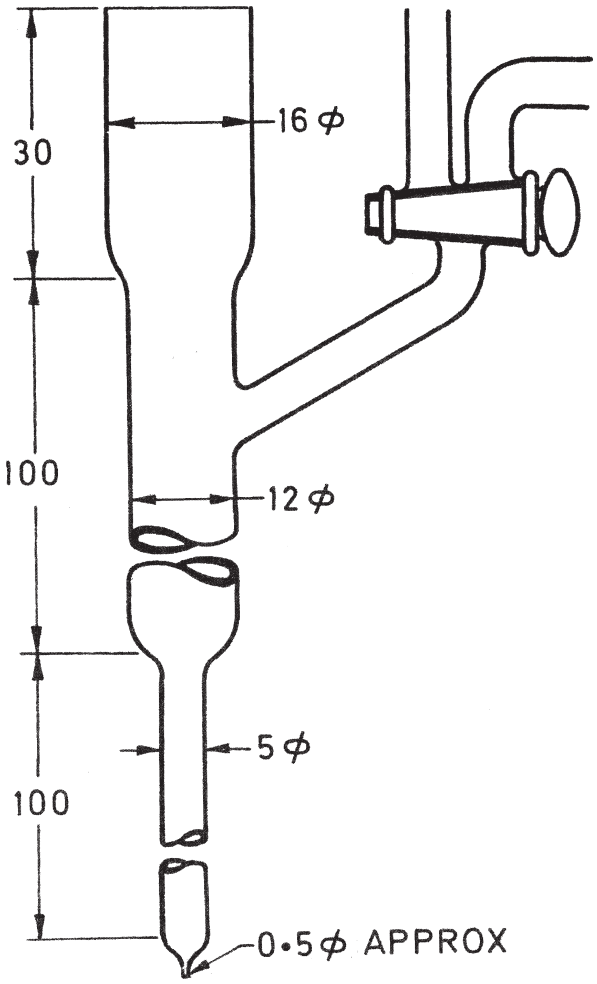


FIG. 1 ASSEMBLY OF CHROMATOGRAPHIC TUBES

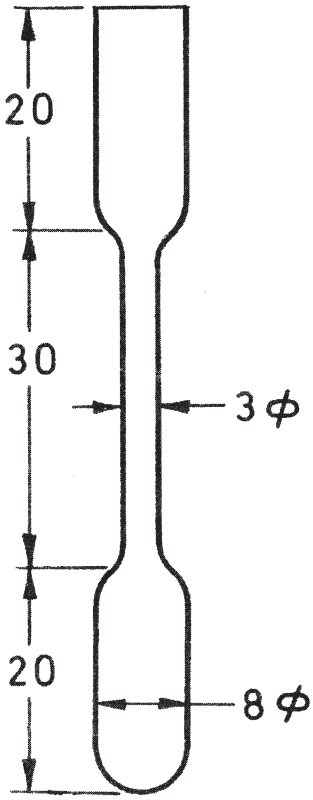


All dimensions in millimetres.

FIG. 2 UPPER TUBE



All dimensions in millimetres.
FIG. 3 LOWER TUBE



All dimensions in millimetres.
FIG. 4 RECEIVING TUBE

C-3.8 Ultra-Violet Spectrophotometer

C-4 PROCEDURE

C-4.1 Sampling

Take a sample of the material weighing approximately 10 g. Such a sample contains a total of about 250 I.U. of vitamin A.

C-4.2 Saponification

Put the sample into the 200 ml round bottom flask and weigh to the nearest 50 mg. Add 8 ml of potassium hydroxide solution and 25 ml ethanol. Heat gently on a steam bath for 1 h or until the saponification is complete, with a reflex condenser attached to the flask. During this procedure, pass a slow current of oxygen free nitrogen through the liquid (*see* Fig. 5).

C-4.3 Extraction of Vitamin A

Add 50 ml water through the condenser and cool the soap solution in tap water. Transfer the solution to a separating funnel, using another 50 ml of water to rinse the flask. Extract the soap solution with successive portions of 100 and 50 ml diethyl ether by shaking. Wash the combined extracts in another separating funnel four times with 50 ml of water (the first time only by twirling, the following three times by gentle shaking). Continue washing if the ether layer is still turbid.

C-4.4 Evaporation of the Solvent

Use the same flask in which the Saponification was carried out. Add the diethyl ether solution in two portions. Heat on the water-bath (80 to 85°C). During the distillation, maintain a weak current of oxygen-free nitrogen until approximately 5 ml is left. Then transfer the residue to a 50 ml conical flask, and rinse with 15 to 20 ml ether. Evaporate the ether with nitrogen, add some acetone, and again evaporate the solvents (*see* Fig. 6).

C-4.4.1 Immediately take up the residue in l-2 ml petroleum ether (if not clear, dry again with acetone), and introduce the solution on top of the upper chromatographic column with as little petroleum ether as possible (about 5 ml).

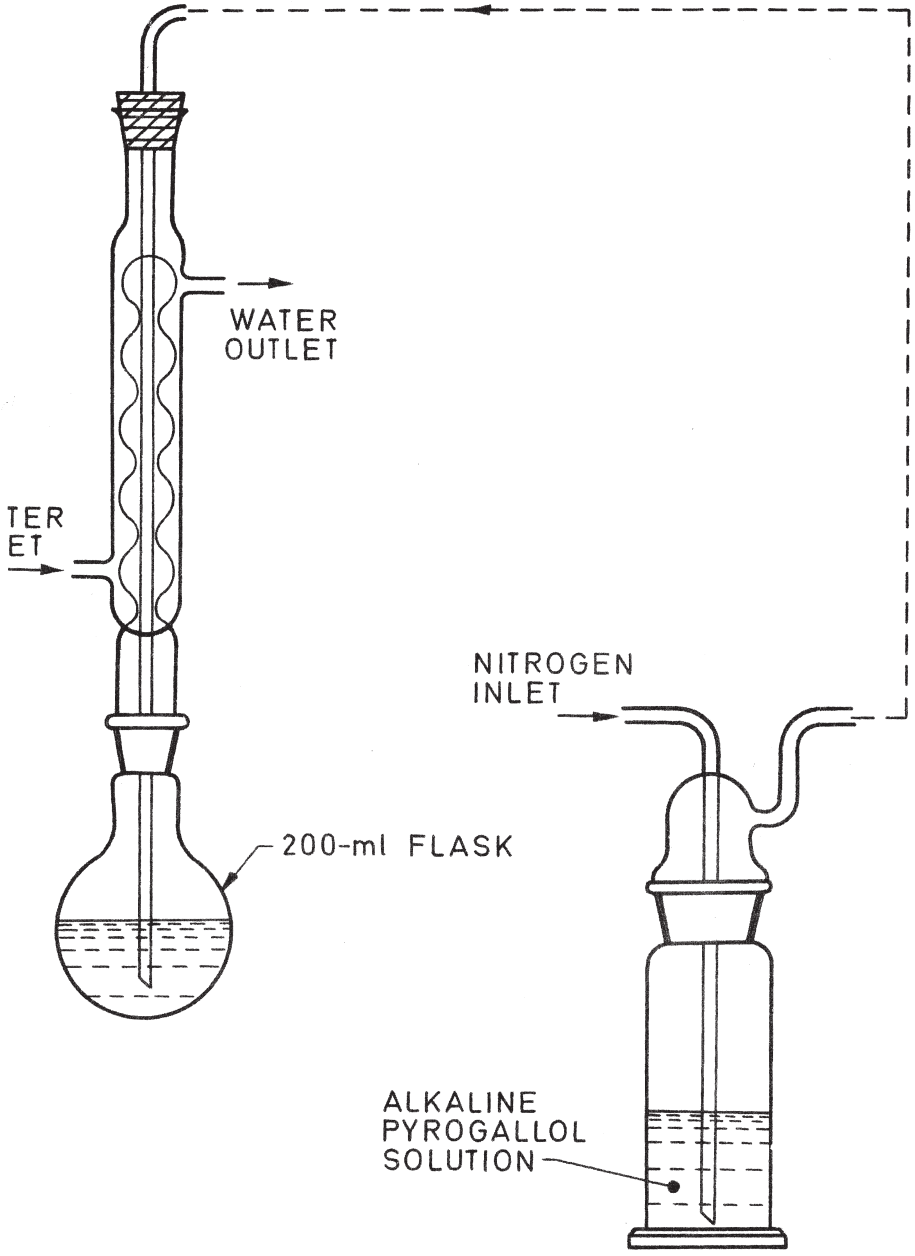


FIG. 5 APPARATUS FOR SAPONIFICATION

C-4.5 Chromatographic Separation of Vitamin A Alcohol

The chromatographic separation is carried out in two stages. For the first stage only the upper column is used (alumina). After the petroleum ether extract has been brought on to the column, this is passed through the adsorbent. Rinse the conical flask with 5 ml petroleum ether and bring this amount on to the column. Rinse the flask with 5 ml portions of petroleum ether containing 4, 8 and 12 percent diethyl ether (*v/v*) respectively, and use the washings for elution process. Discard all the elutes containing substances which are less strongly adsorbed than vitamin A alcohol.

NOTE — Test for vitamin A with antimony trichloride in the elute containing 12 percent diethyl ether. If the reaction is positive (in which case the alumina contains too much water), repeat the determination with a fresh column packing.

C-4.5.1 Then connect the second column (alkaline alumina) and elute with 5 ml each of petroleum ether containing 16, 20 and 24 percent diethyl ether (*v/v*), respectively. Finally, use petroleum ether containing 36 percent diethyl ether (*v/v*), until the vitamin A has been eluted completely. Collect the eluted fractions in the tubes graduated at 1 ml. First thoroughly mix the contents of the each tube in order to obtain a homogeneous solution. Do this by blowing some air bubbles through it by means of the blunt tipped pipette.

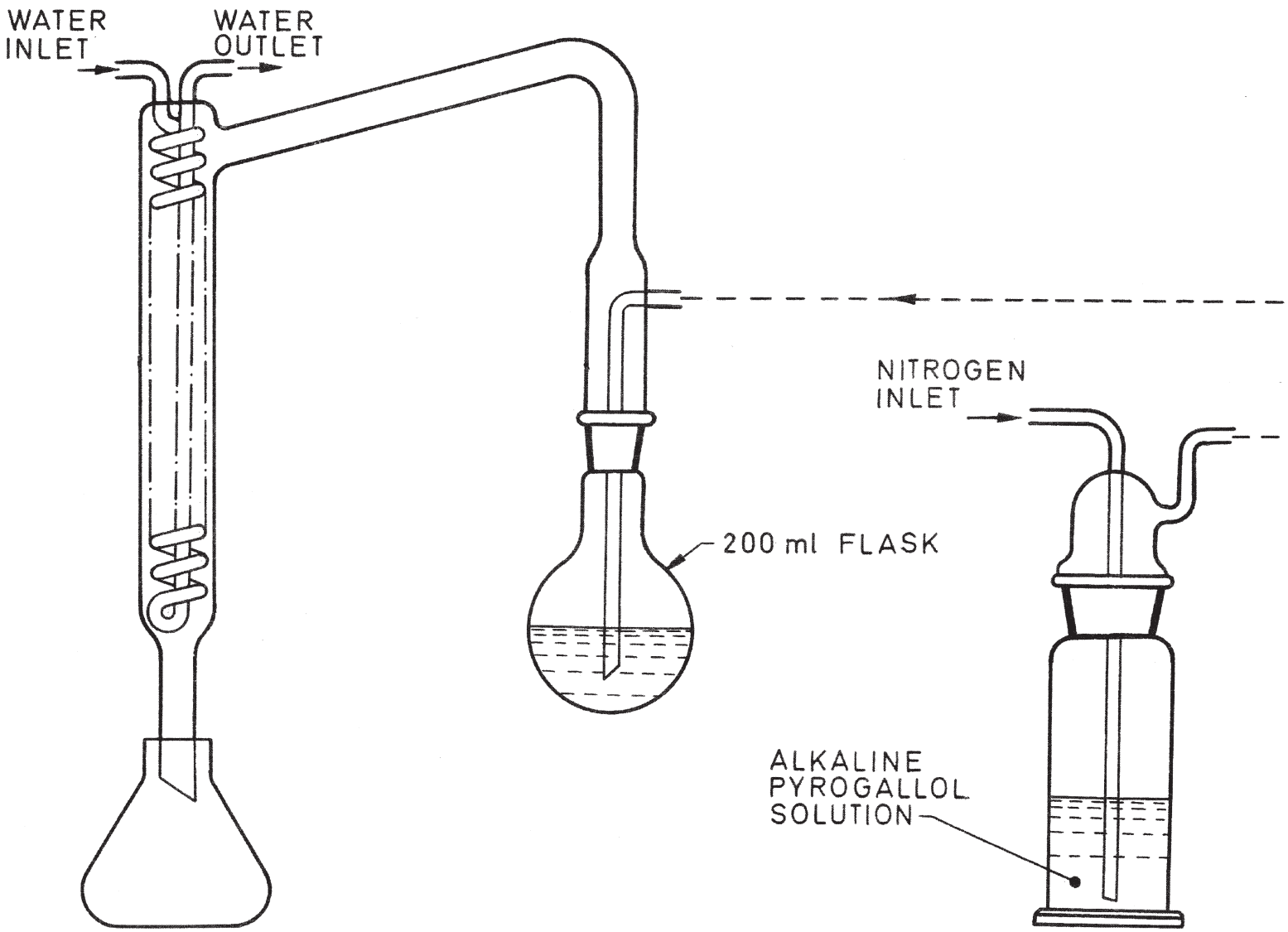


FIG. 6 APPARATUS FOR EVAPORATION

Then with the same pipette, remove from each of the tubes approximately 0.3 ml of the solution and with these samples carry out the Carr-Price spot test (using small test tube) with 0.5 ml antimony trichloride solution and 1 drop of acetic anhydride.

NOTE — Ensure that during elution the columns do not run dry, but at the same time prevent, as much as possible, the various petroleum ether fractions from mixing at the top of the first column. Only very little elute originating from the first column may be present on top of the second column.

C-4.5.1.1 Discard the fractions in which the Carr-Price spot test is negative [(generally those with 16 and 20 percent diethyl ether (*v*/*v*)].

NOTE — Special care should be taken in carrying out Carr-Price spot test, as the reaction is instantaneous (takes 3 to 6 seconds only) and the blue colour developed is very unstable. It is, therefore, recommended that the addition of antimony trichloride reagent should be done quickly, using a blunt tipped pipette.

C-4.6 Spectrophotometric Determination of Vitamin A

Pipette exactly 0.5 ml from each tube in which the Carr-Price spot test is positive. Pool these in the 10 ml graduated flask, make up to the mark with petroleum ether used and mix. Make sure that the absorption at 326 mm of the petroleum ether used does not change when 10 percent diethyl ether is added. Use petroleum ether in the blank cell. Read the optical density at the top of the extinction curve (324 to 326 mm) using a 1 cm Lovibond cell.

NOTE — It is recommended to occasionally compare the shape of the optical density curve between 260 and 370 pm with Morton Stubbs ideal curve. This is done to establish whether the separating capacity of the columns is sufficient. The freshly prepared adsorbents should always be tested in this way.

C-5 CALCULATION

$$\text{Vitamin A (I. U./g)} = \frac{360\,a}{M}$$

where

a = optical density reading at maximum, and

M = mass of sample, in g.

ANNEX D

[*Table* 1, *Sl No*. (v) (b)]

QUALITATIVE DETERMINATION OF VITAMIN A (ANTIMONY TRICHLORIDE METHOD)

D-0 PRINCIPLE

The melted sample is treated with antimony trichloride and observed for appearance of blue colouration which indicates presence of vitamin A.

D-1 REAGENTS

D-1.1 Antimony Trichloride Solution, prepare by dissolving 113.4 g antimony trichloride in 300 to 400 ml of chloroform. Add 5 g of anhydrous calcium chloride and filter while hot. Dilute the filtrate to 500 ml with chloroform.

D-2 PROCEDURE

D-2.1 Take 10 ml of antimony trichloride solution in a test tube and add 15 ml of melted *Vanaspati*.

D-2.2 The material shall be considered to have passed the test, if a blue colouration appears immediately at the interface, indicating the presence of vitamin A.

NOTES

1 Antimony trichloride solution is made in chloroform and this phase is heavier than *Vanaspati*. Therefore, *Vanaspati* should be added to antimony trichloride solution.

2 Special care should be taken in carrying out this test since the reaction is spontaneous and the blue colour developed is very unstable.

ANNEX E

[*Clause* 7.2.1 and *Table* 1, *Sl No*. (vi)]

DETERMINATION OF NICKEL IN *VANASPATI*

E-0 Two methods have been prescribed, the calorimetric method and the atomic absorption spectrometric method. The atomic absorption spectrometric method is the reference method.

E-1 COLORIMETRIC METHOD

E-1.1 Reagents

E-1.1.1 *Concentrated Ammonia*

E-1.1.2 *Sodium Citrate,* ten percent.

E-1.1.3 *Dimethyl Glyoxime,* one percent in ethyl alcohol.

E-1.1.4 *Chloroform*

E-1.1.5 *Ammonia* (0.5 M), 1: 3.

E-1.1.6 *Ammonia,* 1 : 1.

E-1.1.7 *Hydrochloric Acid,* 0.5 M and 1 N.

E-1.1.8 *Sodium Hydroxide,* 2 N.

E-1.1.9 *Potassium Persulphate,* 2 percent.

E-1.1.10 *Standard Nickel Solution*

Dissolve 0.447 9 g of nickel sulphate $NiS0_4.6H_2O$ in water or 0.1 g of 99.9 percent pure nickel in 10 ml of nitric acid and when dissolution is complete, boil gently to expel oxides of nitrogen and dilute to 1 l. This solution contains 0.1 mg/ml of nickel and may be further diluted to 0.01 mg/ml, if necessary.

E-1.1.11 *Perchloric Acid,* 6 percent.

E-1.1.12 *Bromine Water*

E-1.1.13 *Nitric Acid,* 1 N.

E-l.2 Procedure

E-1.2.1 *Preparation of Sample*

Representative sample to give a final solution containing greater than 0.05 but less than 10 µg Ni/ml is weighed into a silica dish a heated to flash N point using a Bunsen burner. When the oil catches tire, Bunsen burner is removed and the oil is allowed to burn in a hood without exhaust. After about 30 min the flame ceases on its own and a very small quantity of residue remains. The residue is heated in low Bunsen flame till smoke ceases, before keeping the sample in the muffle furnace. Ashing is completed in the muffle furnace maintaining the temperature at 550°C. Ashing will be complete within 2 h. Care is needed to take out the sample from the muffle furnace as the ash is very fluffy and quantity is small.

E-1.2.1.1 Dissolve the ash in 1 N nitric acid by warming on the water bath and filter through ash less Whatman filter paper and make up to suitable volume with 1 N nitric acid.

E-1.2.2 *Separation of Nickel by Dimethyl Glyoxime Chloroform Extraction*

To an aliquot containing about 50 to 100 ppm of nickel add 5 ml of 10 percent sodium citrate. Neutralize with concentrated ammonia and add a few drops in excess (*p*H greater than 7.5). Add 2 ml of dimethyl glyoxime (or more as may be required if such copper or cobalt is present, that is, extra volume or 2 ml for each 10 mg of copper and 5 ml for each 10 mg of cobalt). Extract with three 2 to 3 ml portions of chloroform shaking for 30 s each time. Shake the combined chloroform extracts with 0.5 ml of 1 : 3 (0.5) M ammonia. Repeat the wash with another portion of ammonia if much copper or cobalt is present. Shake the ammonia washings with 1 or 2 ml of chloroform and add the latter to the chloroform extract. Carry out reagent blank using the same quantity of reagents.

Return the nickel to the ionic state by shaking with two 5 ml portions of 0.5 M hydrochloric acid, Transfer this solution to a 100 ml volumetric flask and proceed as per calibration.

E-1.2.3 *Colour Development*

E-1.2.3.1 *Method using persulphate for oxidation*

E-1.2.3.1.1 *Calibration*

Transfer 1, 2, 4, 6, 8 and 10 ml of standard nickel solution (1ml - 0.01mgNi) to six 100 ml volumetric flasks. Add 5 ml of 1 N hydrochloric acid. Dilute to 50 ml and add 1 ml of 10 percent sodium citrate, 3 ml of 2 percent potassium persulphate solution, 15 ml of 2 N sodium hydroxide solution and finally 1 ml of 1 percent ethanolic dimethyl glyoxime solution. Heat to 60 to 70°C and keep at the temperature for 5 min. Cool to room temperature and dilute to volume. Measure the absorbance at 465 nm. Plot the photometric readings of the calibration solution against milligrams of nickel present in 100 ml solution.

E-1.2.3.1.2 Develop the colour for sample as described under calibration.

E-1.2.3.2 Method using bromine for oxidation.

E-1.2.3.2.1 *Calibration*

Transfer 1, 2, 4, 6, 8 and 10 ml of standard nickel solution (1 ml - 0.01 mg) to six 100 ml volumetric flasks. Add 2 ml of perchloric acid to each flask. Also prepare a separate reference solution by adding 2 ml of perchloric acid to a 100 ml volumetric flask. Dilute to 50 ml and add successively (swirling the flasks between additions) 10 ml of sodium citrate. 5 ml of bromine water and adjust sufficient ammonia (1 : 1) to bleach the bromine colour. Add 3 ml of ammonia (1 : 1) in excess and cool to room temperature. Add 3 ml of dimethyl glyoxime solution, dilute to the mark and mix (the addition of bromine water, ammonia and dimethyl glyoxime). Transfer a suitable quantity of the solution to an absorption cell, cool and measure the absorbance to 530 nm. Plot the photometric readings of the calibration solution against milligrams of nickel per 100 g of solution.

E-1.2.3.2.2 Develop the colour for sample as described under calibration and read the absorbance against reagent blank.

E-1.2.4 *Calculation*

$$\text{Concentration of nickel, in sample (ppm)} = \text{Measured concentration (from calibration curve)} \times \frac{\text{Dilution factor}}{\text{Mass of sample, in g}}$$

E-2 ATOMIC ABSORPTION SPECTROMETRIC METHOD

E-2.1 Reagents

E-2.1.1 *Stock Nickel Solution* — Dissolve 1 g of 99.99 percent nickel in minimum volume of 1 + 1 HNO_3 and dilute to one litre with 1 percent nitric acid.

E-2.1.2 *Nitric Acid* — 1 N.

E-2.2 Procedure

E-2.2.1 *Preparation of Sample*

Representative sample to give a final solution containing greater than 0.05 but less than 10 µg Ni/ml is weighed into a silica dish and heated to flash point using a Bunsen burner. When the oil catches fire, Bunsen burner is removed and the oil is allowed to burn in a hood without exhaust. After about 30 min the flame ceases on its own and a very small quantity of residue remains. The residue is heated in low Bunsen flame till smoke ceases, before keeping the sample in the muffle furnace. Ashing is completed in the muffle furnace maintaining the temperature at 450°C. Ashing will be complete within 2 h. Care is needed to take out the sample from the muffle furnace as the ash is very fluffy and quantity is small.

E-2.2.1.1 Dissolve the ash in 1 N nitric acid by warming on the water-bath and filter through ashless Whatman filter paper and make up to suitable volume with 1 N nitric acid.

E-2.2.2 *Calibration of Atomic Absorption Spectrometer*

Calibrate the instrument with 0.2, 0.4, 0.8, 1.6, 2.0, 4.0, 8.0 and 10 mg/ml standard nickel solution by setting the wavelength of 232 nm using air acetylene flame and 1 N nitric acid as blank. The absorbance is plotted against concentrate.

E-2.2.3 *Measurement*

Run the sample solution into the instrument and calculate the concentration of nickel in the sample.

E-2.2.4 *Calculation*

$$\text{Concentration of nickel, in sample (ppm)} = \text{Measured concentration} \times \frac{\text{Dilution factor}}{\text{Mass of sample, in g}}$$

CAUTION — Boron trifluoride may be fatal, if inhaled.

ANNEX F

[*Table* 1, *Sl No*. (vii)]

DETERMINATION OF TRANS FATTY ACIDS

F-1 PRINCIPLE

Fat and fatty acids are extracted from food by hydrolytic methods (acidic hydrolysis for most products, alkaline hydrolysis for dairy products, and combination for cheese). Pyrogallic acid added to minimize oxidative degradation of fatty acids during analysis. Triglyceride, triundecanoin ($C_{11:0}$), is added as internal standard. Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMEs) using BF_3 in methanol. FAMEs are quantitatively measured by capillary gas chromatography (GC) against $C_{11:0}$ internal standard. Total fat is calculated as sum of individual fatty acids expressed as triglyceride equivalents. Saturated and monounsaturated fats are calculated as sum of respective fatty acids. Monounsaturated fat includes only *cis* form.

F-2 APPARATUS

F-2.1 Gas Chromatograph (GC), equipped with hydrogen flame ionization detector, capillary column, split mode injector, oven temperature programming sufficient to implement a hold ramp hold sequence. Operating conditions: temperature (°C): injector, 225; detector, 285; initial temp, 100 (hold 4 min); ramp, 3°C/ min; final temperature 240; hold 15 min; carrier gas, helium; flow rate, 0.75 ml/ min; linear velocity, 18 cm/s; split ratio, 200:1.

F-2.2 Capillary Column, separating the FAME pair of adjacent peaks of $C_{18:3}$ and $C_{20:1}$ and the FAME trio of adjacent peaks of $C_{22:1}$, $C_{20:3}$, and $C_{20:4}$ with a resolution of 1.0 or greater. SP2560 100 m × 0.25 mm with 0.20 mm film is suitable.

F-2.3 Mojonnier Flasks

F-2.4 Stoppers, synthetic rubber or cork.

F-2.5 Mojonnier Centrifuge Basket

F-2.6 Hengar Micro Boiling Granules

F-2.7 Baskets, aluminum and plastic.

F-2.8 Shaker Water Bath, maintaining 70-80°C.

F-2.9 Steam Bath, supporting common glassware.

F-2.10 Water Bath, with nitrogen stream supply, maintaining 40 ± 5°C.

F-2.11 Wrist Action Shaker, designed for Mojonnier centrifuge baskets.

F-2.12 Mojonnier Motor Driven Centrifuge — Optional; maintaining 600 × g.

F-2.13 Gravity Convection Oven — Maintaining 100 ± 2ºC.

F-2.14 Vortex Mixer

F-2.15 Gas Dispersion Tubes, 25 mm, porosity "A", extra coarse 175 mm.

F-2.16 Three Dram Vials, about 11 ml.

F-2.17 Phenolic Closed Top Caps, with polyvinyl liner, to fit vials.

F-2.18 Teflon/Silicone Septa, to fit vials.

F-3 REAGENTS

F-3.1 Pyrogallic Acid

F-3.2 Hydrochloric Acid, 12 M and 8.3 M. To make 8.3 M HCL add 250 ml 12 M HCl to 10 ml H_2O. Mix well Store at room temperature (20-25°C).

F-3.3 Ammonium Hydroxide, 58 percent (*w/w*).

F-3.4 Diethyl Ether, purity appropriate for fat extraction.

F-3.5 Petroleum Ether, anhydrous.

F-3.6 Ethanol, 95 per cent (*v/v*).

F-3.7 Toluene, nanograde.

F-3.8 Chloroform

F-3.9 Sodium Sulphate, anhydrous.

F-3.10 Boron Trifluoride Reagent, 7 percent BF_3 (*w/w*) in methanol, made from commercially available 14 percent BF solution. Prepare in the hood.

'**CAUTION** — BORON TRIFLUORIDE MAY BE FATAL IF INHALED'

F-3.11 Diethyl Ether-Petroleum Ether Mixture, 1 + 1 (*v/v*).

F-3.12 Triglyceride Internal Standard Solution, $C_{11:0}$-triundecanoin; 5.00 mg/ml in $CHCl_3$. Accurately weigh 2.50 g $C_{11:0}$-triundecanoin into 500 ml volumetric flask. Add ca 400 ml $CHCl_3$ and mix until dissolved. Dilute to volume with $CHCl_3$. Invert flask at least 10 additional times. Triglyceride internal standard solution is stable up to 1 month when stored in refrigerator (2-8°C).

F-3.13 Fatty Acid Methyl Esters (FAMEs) Standard Solutions

F-3.13.1 *Mixed FAMEs Standard Solution*, reference mixture containing series of FAMEs, including $C_{18:1}$ *cis* and *trans* (available commercially, or equivalent). To prepare mixed FAMEs standard solution break top of glass vial, open and carefully transfer contents of to 3-dram glass vial. Wash original vial with hexane to ensure complete transfer and add washings to 3-dram glass vial. Dilute to about 3 ml with hexane.

F-3.13.2 *$C_{11:0}$ FAME Standard Solutions*, $C_{11:0}$-Undecanoic methyl ester in hexane. Use only in preparation of individual FAME standard solutions (*see* **F-3.13.3**). To prepare $C_{11:0}$ FAME standard solutions, break top of glass vial open and carefully transfer contents to 50 ml volumetric flask. Wash original vial with hexane to ensure complete transfer and add washings to 50 ml volumetric flask. Dilute to volume with hexane. $C_{11:0}$ FAME standard solution is stable up to 1 week when stored at 0ºC.

F-3.13.3 *Individual FAME Standard Solutions,* standard solutions of each of following FAMEs : $C_{4:0}$-tetranoic methyl ester, $C_{6:0}$-hexanoic methyl ester, $C_{8:0}$-octanoic methyl ester, $C_{10:0}$-decanoic methyl ester, $C_{12:0}$-dodecanoic methyl ester, $C_{13:0}$-tridecanoic methyl ester, $C_{14:0}$-tetradecanoic methyl ester, $C_{14:1}$-9-tetradecenoic methyl ester, $C_{15:0}$-pentadecanoic methyl ester, $C_{15:1}$-10-pentadecenoic methyl ester, $C_{16:0}$-hexadecanoic methyl ester, $C_{16:1}$-9-hexadecenoic methyl ester, $C_{17:0}$-heptadecanoic methyl ester, $C_{17:1}$-10-heptadecenoic methyl ester, $C_{18:0}$-octadecanoic methyl ester, $C_{18:1}$-9-octadecenoic methyl ester, $C_{18:2}$-9, 12-octadecadienoic methyl ester, $C_{18:3}$-9.12,15-octadecatrienoic methyl ester, $C_{20:2}$-11,14-eicosadienoic methyl ester, $C_{20:3}$-11,14,17-eicosatrienoic methyl ester, and $C_{22:0}$-docosanoic methyl ester. Prepare individual FAME standard solutions as follows: Break top of glass vial open and carefully transfer contents to 3 dram glass vial. Wash original vial with hexane to ensure complete transfer and add washings to 3 dram glass vial. Add 1.0 ml $C_{11:0}$ FAME standard solution (*see* **F-3.13.2**) and dilute to total volume of about 3.0 ml with hexane. Individual FAME standard solutions are stable up to 1 week when stored in refrigerator (2-8ºC).

F-4 EXTRACTION OF FAT

Accurately weigh ground and homogenized test portion (containing ca 100-200 mg fat) into labeled Mojonnier flask. Force material into flask as far as possible. Add ca 100 mg pyrogallic acid (*see* **F-3.1**), and 200 ml triglyceride internal standard solution (*see* **F-3.12**). Add a few boiling granules to flask. Add 2.0 ml ethanol and mix well until entire test portion is in solution. Add 10.0 ml 8.3 M HCl and mix well. Place flask into basket in shaking water bath at 70-80ºC set at moderate agitation speed for 40 min. Mix contents of flask on Vortex mixer every 10 min to incorporate particulates adhering to sides of flask into solution. After digestion, remove flask from bath and allow to cool to room temperature (20-25ºC). Add enough ethanol to fill bottom reservoir of flak and mix gently.

F-5 METHYLATION

Dissolve extracted fat residue in 2-3 ml chloroform and 2-3 ml diethyl ether. Transfer mixture to 3 dram glass vial and then evaporate to dryness in 40ºC water bath under nitrogen stream. Add 2.0 ml 7% BF, reagent (*see* **F-3.10**) and 1.0 ml toluene (*see* **F-3.7**). Seal vial with screwcap top containing Teflon/silicone septum. Heat vial in oven 45 min at 100ºC. Gently shake vial ca every 10 min.

NOTE — Evaporation of liquid from vials indicates inadequate seals: if this occurs, discard solution and repeat the entire procedure.

Allow vial to cool to room temperature (20-25ºC). Add 5.0 ml H_2O, 1.0 ml, hexane, and ca 1.0 g Na_2SO_4 (**F-3.9**). Cap vial and shake 1 min. Allow layers to separate and then carefully transfer top layer to another vial containing ca 1.0 g Na_2SO_4.

NOTE —Top layer contains FAMEs including FAME of triglyceride internal standard solution.

F-6 GC DETERMINATION

Relative retention time (*versus* FAME of triglyceride internal standard solution) and response factors of individual FAMEs can be obtained by GC analysis of individual FAME standard solution and mixed FAME standard solution. Inject ca 2 µl each of individual FAME standard solutions and 2µl of mixed FAMEs standard solution. Use mixed FAMEs standard solution to 0ptimize chromatographic response before injecting any test solutions. After all chromatographic conditions have been optimized, inject test solutions from **F-5**.

NOTE — With matrixes of unknown composition, it may be necessary to analyze test portion without addition of internal standard to ensure against interferences. Should interfering peak be found, the area of C_{11} internal standard peak must be corrected before performing calculations. Use 2.0 ml chloroform instead of internal standard solution.

F-7 CALCULATIONS

Calculate retention times for each FAME in individual FAMEs standard solutions (*see* **F-3.13.3)**, by subtracting retention time of $C_{11:0}$ peak from retention time of fatty acid peak. Use these retention times to identify FAMEs in mixed FAMEs standard solution. Use additional FAME solutions (from the same supplier) when necessary for complete FAME identity verification.

F-7.1 Calculate response factor (R_i) for each fatty acid *i* as follows:

$$R_i = \frac{Ps_{\mathrm{i}}}{Ps_{\mathrm{C11:0}}} \times \frac{W_{\mathrm{C11:0}}}{W_{\mathrm{i}}}$$

where

Ps_{i} = peak area of individual fatty acid in mixed FAMEs standard solution.

$Ps_{\mathrm{C11:0}}$ = peak area of $C_{11:0}$ fatty acid in mixed FAMEs standard solution

$W_{\mathrm{C11:0}}$ = weight of internal standard in mixed FAMEs standard solution

W_{i} = weight of individual FAME in mixed FAMEs standard solution.

NOTE — Peaks of known identity with known relative retention times are listed in Table 3

Table 3 Retention Time of Fatty Acids and Methyl Ester (FAME)

(*Clause* F-7.1)

Fatty Acid	Retention Time, min	Relative Retention Times (to 11:0 Internal Standard)
4:0 Butyric	10.49	0.46
6:0 Caproic	12.36	0.54
8:0 Caprylic	15.69	0.68
10:0 Capric	20.39	0.89
11:0 Undecanoic	22.99	1.00
12:0 Lauric	25.58	1.11
13:0 Tridecanoic	28.15	1.22
14:0 Myristic	30.65	1.33
14:1 Myristoleic	32.63	1.42
14:1 *trans*-Myristelaidic	32.01	1.39
15:0 Pentadecenoic	33.04	1.44
15:1 Pentadecenoic	34.98	1.52
16:0 Palmitic	35.41	1.54
16:1 *trans*-Palmitelaidic	36.39	1.58
16:1 Palmitoleic	36.88	1.60
17:0 Margaric	37.54	1.63
17:1 Margaroleic	38.92	1.69
18:0 Stearic	39.78	1.73
18:1 *trans* 6-Petroseienic	40.50	1.76
18:1 *trans*-Elaidic	40.61	1.77
18:1 *trans* 11-Vaccenic	40.72	1.77
18:1 Petroseilenic	40.90	1.78
18:1 Oleic	40.99	1.78
18:1 Vaccenic	41.18	1.79
18:1 Octadecenoic	41.54	1.81
18:2 *trans*-Linoleaidic	41.69	1.81
18:2 *trans* 9-Linolelaidic	42.11	1.83
18:2 *trans*12-Linolelaidic	42.53	1.85
18:2 Linoleic	42.87	1.86
20:0 Arachidic	43.75	1.90
18:3 g-Linolenic	44.25	1.92
20:1 Eicosenic *cis* 5	44.42	1.93
20: 1 Eicosenic *trans* 11	44.45	1.93
20:1 Eicosenic *cis* 8	44.67	1.94
20:1 Eicosenic *cis* 11	44.82	1.95
20:1 Eicosenic *cis* 13	44.99	1.96
18:3 Linolenic	45.02	1.96
18:2 Linoleic-conjugated	45.35	1.97
18:2 Linoleic-conjugated	45.40	1.97
21:0 Heneicosanoic	45.69	1.99
18:2 Linoleic-conjugated	46.18	2.01
18:4 Octadectetraenoic	46.39	2.02
20:2 Eicosadienoic	46.65	2.03
22:0 Behenic	47.46	2.06
20:3 g-Eicosatrienoic	47.94	2.09
22:1 Cetoleic	48.27	2.10
22:1 Erucic	48.50	2.11
20:3 Eicosatrienoic	48.68	2.12
20:4 Arachidonic	48.94	2.13
23:0 Tricosanoic	49.22	2.14
22:2 Docosadienoic	50.17	2.18
24:0 Lignoceric	50.79	2.21
20:5 Eicosapentaenoic	50.96	2.22
24:1 Nervonic	51.92	2.26
22:3 Docosatrienoic	51.98	2.26
22:4 Docosatetraenoioc	52.28	2.27
22:5 Docosapentaenoic	54.75	2.38
22:6 Docosahexaenoic	55.82	2.43

F-7.2 Calculate amount of individual (triglyccrides) (W_{TG}) in test sample as follows:

$$W_{\mathrm{FAMEi}} = \frac{Pt_{\mathrm{i}} \times Wt_{\mathrm{C11:0}} \times 1.006\ 7}{Pt_{\mathrm{C11:0}} \times R_{\mathrm{i}}}$$

$$W_{\mathrm{TGi}} = W_{\mathrm{FAMEi}} \times f_{\mathrm{TGi}}$$

where

Ri = response factor of fatty acid *i*;

Pt_{i} = peak area of fatty acid *i* in test portion;

$Wt_{\mathrm{C11:0}}$ = weight of $C_{11:0}$ internal standard added to test portion, g;

1.006 7 = conversion of internal standard from triglyceride to FAME;

$Pt_{\mathrm{C11:0}}$ = peak area of $C_{11:0}$ internal standard in test portion; and

f_{TGi} = conversion factor for FAMEs to triglycerides for individual fatty acids (*see* Table 4).

NOTE —If procedure is followed exactly, $Wt_{\mathrm{C11:0}}$ should be 0.010 g)

Table 4 Factors (f_{TG}) for Conversion of FAMEs to Triglyceride Equivalents

(*Clause* F-7.2)

Fatty Acid	f_{FAi} [1)]	Tri/FAME (f_{TGi})[2)]
4:0 Butyric	0.8627	0.9868
6:0 Caproic	0.8923	0.9897
8:0 Caprylic	0.9114	0.9915
10:0 Capric	0.9247	0.9928
11:0 Undecanoic	0.9300	0.9933
12:0 Lauric	0.9346	0.9937
13:0 Tridecanoic	0.9386	0.9941
14:0 Myristic	0.9421	0.9945
14:1 Tetradecenenoic	0.9417	0.9944
15:0 Pentadecenoic	0.9453	0.9948
15:1 Pentadecenoic	0.9449	0.9947

Fatty Acid	f_{FAi} [1)]	Tri/FAME (f_{TGi})[2)]
16:0 Palmitic	0.9481	0.9950
16:1 Hexadecenoic	0.9477	0.9950
17:0 Margaric	0.9507	0.9953
17:1 Margaroliec	0.9503	0.9952
18:0 Stearic	0.9530	0.9955
18:1 Octadecenoic	0.9527	0.9955
18:2 Octadecdieoic	0.9524	0.9954
18:3 Linolenic	0.9520	0.9954
18:4 Octadectetraenoic	0.9517	0.9954
20:0 Arachidic	0.9570	0.9959
20:1 Eicosenic	0.9568	0.9959
20:2 Eicosadienoic	0.9565	0.9958
20:3 Eicosatrienoic	0.9562	0.9958
20:4 Arachidonic	0.9560	0.9958
20:5 Eicosapentaenoic	0.9557	0.9958
21:0 Heneicosanoic	0.9588	0.9961
22:0 Behenic	0.9604	0.9962
22:1 Docosaenoic	0.9602	0.9962
22:2 Docosadienoic	0.9600	0.9962
22:3 Docosatrienoic	0.9598	0.9961
22:4 Docosatetraenoic	0.9595	0.9961
22:5 Docosapentaenoic	0.9593	0.9961
22:6 Docosahexaenoic	0.9590	0.9961
23:0 Tricosanoic	0.9620	0.9964
24:0 Lignoceric	0.9963	0.9965
24:1 Nervonic	0.9632	0.9965

[1)] f_{FAi} is the conversion factor for conversion of FAMESs to corresponding fatty acids.
[2)] f_{TGi} is the conversion factor for conversion of FAMEs to triglycerides for individual fatty acids.

F-7.3 Calculate weight of each fatty acid *(W_i)* as follows:

$$W_i = W_{FAMEi} \times f_{FAi}$$

where

f_{FAi} = conversion factors for conversion of FAMEs to their corresponding fatty acids *(see* Table 4).

F-7.4 Calculate percent of *trans* fat in test sample [*w/w*; expressed as sum of only *trans* fatty acids ($C_{14:1}$ Trans Myristelaidic + $C_{16:1}$ Trans Palmitelaidic + $C_{18:1}$ Trans 6 Petroselenic + $C_{18:1}$ Trans Elaidic + $C_{18:1}$ Trans 11 Vaccenic + $C_{18:2}$ Trans Linolelaidic + $C_{18:2}$ Trans 9- Linolelaidic + $C_{18:2}$ Trans 12-Linolelaidic + $C_{18:3}$ Trans Linolenic + $C_{20:1}$ Eicosenic Trans 11)] as follows:

$$\text{Transfat, percent} = (\Sigma_{transfat}\, W_i / W_{test\ portion}) \times 100\ \%$$

NOTE — Test samples containing hydrogenated fat will yield complicated chromatograms due to large number of isomers formed during hydrogenation process. One general indication of hydrogenation is presence of $C_{18:1}$ *trans* peak(s). *trans* peaks elute prior to *cis,* therefore, include all peaks between $C_{18:1}$ *cis* and $C_{18:2}$ *cis,cis* in calculation of $C_{18:2}$ peak area. Often $C_{18:1}$ *trans* "peak" consists of broad series of peaks [due to positional isomers from hydrogenation]; include all of these in $C_{18:1}$ *trans* peak area.

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